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Comments on the December 31, 1998, External Review Draft of the EPA/NCEA Document, "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information"

Submitted to the External Peer-Reviewers

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I. Introduction

The External Review Draft[1] describes the derivation of a revised RfD for perchlorate based on animal data. In Section II of this commentary, the derivation of this revised RfD is critiqued and suggestions are offered for a more physiologically integrated approach to using the animal data as the basis of the RfD.

The RfD derivation presented in the External Review Draft is essentially devoid of reference to available information on safe levels of human exposure to perchlorate. More to the point, additional human data are currently being gathered; these address the effects of short-term perchlorate exposures on thyroidal uptake of iodide, serum levels of thyroid hormones, and the kinetics of perchlorate elimination. Preliminary results are expected within the month, and final results a few months later. Section III briefly suggests how the human data could be utilized to ensure greater relevance to humans in the derivation of a revised RfD.

II. Animal Data As the Basis of the RfD

A. Choice of the Critical Study and Critical Endpoint

In the External Review Draft, EPA/NCEA evaluated the results of six new, animal-toxicity bioassays. Final study reports were available for four of these (Caldwell *et al.*'s 14-day study in rats, Springborn Laboratories' 14-day and 90-day study in rats, a neurodevelopmental study in rats, and a developmental toxicity study in rabbits), while only incomplete study results were available for the other two (a two-generation reproductive study in rats and an immunotoxicity study in mice). Based upon the results of the new studies, including some reanalyses of raw data by EPA/NCEA, critical effects were chosen and a principal study was identified. According to the External Review Draft (p. 6-2), "the overwhelming weight of the evidence from these studies support[s] the use of the hormone and thyroid histology evidence as the choice for critical effects." The critical study chosen was the neurodevelopmental study. The dose-response for follicular hypertrophy and decreased lumen size in postnatal-day-5 (PND5) pups (as revealed by standard histopathology techniques) formed the basis of the RfD derivation. To quantify the dose-response for these endpoints, EPA/NCEA performed contingency-table analyses that allowed severity and incidence to be considered together. For both sexes combined, EPA/NCEA found that the lowest dose tested, 0.1 mg/kg-day, produced a statistically significant increase in the incidence and severity of follicular-cell hypertrophy (*i.e.*, increased cellular height and/or diameter) and decreased size of the follicular-cell lumen. Based on these results, the lowest-observed-adverse-effect level (LOAEL) for the critical effect in the principal study was determined to be 0.1 mg/kg-day.

EPA/NCEA has made a good case for focusing on the rat neurodevelopment study, particularly the PND5 data, above all other animal data considered. The new animal bioassays support the premise that the thyroid is the most sensitive target organ. Furthermore, rats exposed to perchlorate *in utero* are, as expected, more susceptible to changes in follicular-cell morphology than are weanling rats exposed for 14 or 90 days. The question remains, however, whether such changes are adverse in and of themselves. Are these changes merely histologically identifiable or are they indicative of a pathological process?

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[1] "External Review Draft" is used herein as an abbreviation for the EPA/NCEA/ORD document, "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information."

B. Physiological Significance of Follicular Hypertrophy and Decreased Lumen Size

EPA/NCEA has argued, persuasively, that an exposure which produces transient effects of no toxicological significance to the adult animal might, on the other hand, produce permanent damage to the fetus or neonate. If exposure to a substance at a given dose produces irreversible histopathological changes, then it would be reasonable to conclude that a toxic threshold has been exceeded. However, no evidence for a permanent lesion has been found in rat pups exposed *in utero* and *via* breast milk to a maternal perchlorate dose as high as 10 mg/kg-day. The increased incidence of follicular-cell hypertrophy observed on PND5 in the 0.1 and 1.0 mg/kg-day dose groups was no longer evident in pups sacrificed on PND10. In pups with dosing discontinued on PND10, levels measured on PND22 were elevated relative to control levels only in the 1.0 mg/kg-day group; however, the statistical significance was not given and the absence of an effect in the 3.0 and 10 mg/kg-day groups argues against the relevance of this elevation. Table 1 shows the incidence data as given in Tables 5-3 (p. 5-29) and 5-4 (p. 5-30) of the External Review Draft.

EPA/NCEA has classified increased follicular-cell size (hypertrophy) and decreased follicular-cell lumen size as adverse effects. Indeed, there might be circumstances when the histological findings in question are corollary to an underlying pathology. But EPA/NCEA has not provided convincing evidence that such cellular changes, in and of themselves, fall outside the realm of physiologically normal thyroid morphology.

Table 1. Incidence Ratio of Any Evidence of Follicular-Epithelial-Cell Hypertrophy Among Rat Pups in the 1998 Neurodevelopmental Study of Perchlorate by Argus Laboratories, as Determined by Standard Histology

		Perchlorate Dose to the Dams (mg/kg-day)			
Time of Sacrifice	**Control**	**0.10**	**1.0**	**3.0**	**10**
PND5	0.25 (3/12)	0.67 (8/12)	0.75 (9/12)	0.67 (8/12)	1.00 (12/12)
PND10	0.40[a]	0.40[a]	0.40[a]	1.00[a]	1.00[a]
PND22[b]	0.52[a]	0.48[a]	0.68[a]	0.52[a]	0.48[a]

[a] The External Review Draft did not provide the absolute number of animals examined.

[b] Perchlorate exposure discontinued on PND10.

C. Correlation Analyses (Figures 6-3 to 6-16 of the External Review Draft)

In the External Review Draft, EPA develops a toxicologic mode-of-action model "proposed to map the relationships between external dose, internal dose, the biologically effective dose, and altered structural and functional parameters of established relevance to risk assessment" (p. 6-10.) Asserting that "the earliest biological effect, changes in thyroid and pituitary hormones" is the precursor for potential adverse outcomes, EPA notes, "The difficulty in designating an effect level for these perturbations, however, was in the degree of change to designate as adverse." Thyroid hormone/thyroid histology correlation analyses were used to "further support the mode-of-action mapping" model (p. 6-12). The analyses consist of the paired comparisons outlined in Table 2. EPA's hypothesis is that positive correlations for T3 *vs.* T4 and negative correlations for T3 (or T4)

vs. TSH "are expected if these perturbations are affecting thyroid economy." The hypothesis is extended further: "Positive correlations between TSH and thyroid histopathology are expected, whereas T3 or T4 would be correlated negatively (inversely) with thyroid histopathology." Note that the use of the word "histopathology" in this context suggests the unwarranted assumption that the observed histological changes are indeed pathological in nature.

There is no doubt that, in most cases, the analyses revealed statistically robust correlations of the expected sign. The question is whether the results were skewed in favor of the relationships corresponding to higher perchlorate doses. In other words, would the same correlations be found if only the parameter values corresponding to perchlorate doses of, say, 1 mg/kg-day and below were included in the analyses? In the absence of access to the original data, much can be gleaned by visual inspection of figures 6-3 to 6-16. For T3 *vs.* T4, a positive correlation seems to be present even at the lower perchlorate doses. For T4 *vs.* TSH, there is evidence for and against a negative correlation. For the rank order of T4 or TSH *vs.* histopathological severity, it is doubtful that any statistically meaningful correlation would be found at lower doses.

- In Caldwell *et al.*'s 14-day rat study, even if doses above 1.1 mg/kg-day are ignored, an inverse relationship between T4 and TSH is evident upon visual inspection of Fig. 6-3 (p. 6-15). However, this finding contradicts the results of Springborn Laboratories for rats exposed likewise for 14 days (Fig. 6-8, p. 6-20) or of Argus Laboratories for PND5 rats following exposure *in utero* and *via* breast milk (Fig. 6-12, p. 6-24). In the latter two studies, removal of the 10 mg/kg-day data (leaving 1.0 mg/kg-day as the highest dose) would yield a *positive* relationship between T4 and TSH, *i.e.*, opposite to what the EPA/NCEA model predicts. For the remaining analyses of T4 *vs.* TSH (Figs. 6-6, 6-10, and 6-15, pp. 6-18, 6-22, and 6-27), visual inspection of the figures is insufficient to allow firm prediction of the consequences of removal of the data corresponding to perchlorate doses above 1 mg/kg-day; for these analyses, direct analysis of the raw data would be required.
- With respect to the analyses of T4 or TSH rank-order *vs.* severity classification for hypertrophy or decreased lumen size (as determined by standard histopathology), visual inspection of Caldwell *et al.*'s 14-day rat data (Figs. 6-4 and 6-5, pp. 6-16 and 6-17) does not allow prediction of the consequences of removal of the data for higher perchlorate doses. However, visual inspection of Springborn Laboratories' 14-day/90-day combined (Fig. 6-7, p. 6-19) or 14-day (Fig. 6-9, p. 6-21) data in rats indicates that, in both cases, the correlations are clearly dependent on the high-dose group (100 or 10 mg/kg-day). Thus, over perchlorate dose ranges of 0 to 30 mg/kg-day for the 14-day/90-day combined data and 0 to 1mg/kg-day for the 14-day data there appears to be no correlation of the severity of hypertrophy or decreased lumen size with the rank order of either T4 or TSH. To definitely confirm (or disprove) this assertion, one would have to perform sequential statistical analyses: removing the top dose, then the next highest dose, and so on, to see what remains of the relationship between T4 or TSH rank order and follicular-cell morphology after each round of data removal. Performance of this task would require access to the raw data.

If there is no correlation between the thyroid hormones T4 or TSH and thyroid histopathology (follicular hypertrophy or decreased lumen size) at perchlorate doses of 1.0 mg/kg-day and below, it is not useful to argue that such correlations support a model in which thyroid hormone changes *of any magnitude* are considered to be potentially causal to histological changes. It is far more likely that, even in an animal as sensitive as the rat, some fluctuations in T3, T4, and TSH are tolerated without consequent alteration of follicular-cell morphology.

D. Historical Control Data on Serum T3 and T4 in Rats

In the External Review Draft, EPA/NCEA examines the relationship between perchlorate dose and thyroid hormone levels. Although statistical analysis of the dose-response is straightforward, lingering questions remain concerning the physiological significance of small, statistically significant changes. One means of addressing such concerns is to examine the historical data base for thyroid hormones in the control groups of animal bioassays. If a given dose of perchlorate produces a change that falls within the normal range of thyroid-hormone levels seen in animal-bioassay control groups, it would seem unreasonable to argue that such a response is part of a continuum leading to adverse effects.

Using data from bioassays sponsored by the National Toxicology Program (NTP), Dr. Greg Travlos of the National Institutes of Environmental Health Sciences (NIEHS) has assembled a data base of blood-chemistry parameter values found in control animals at the 13-week sacrifice. After excluding data considered by Dr. Travlos to be invalid or unreliable, there were data from thirteen bioassays for T3 and sixteen for T4. At this time, the entire data base on TSH is considered to be unreliable, either because of standardization issues, measurement errors, reporting errors, or some combination thereof (Dr. Greg Travlos, personal communication).

Figures 1 and 2 present mean values of serum total T3 and T4 in control-group rats at the 13-week termination point in NTP-sponsored studies. Also shown in Figures 1 and 2 are serum total T3 and T4 levels measured in the 90-day, drinking-water exposure study of perchlorate in rats. Note that mean serum T3 levels were higher in both male and female perchlorate controls than in any of the NTP control groups. Mean serum T4 levels in the perchlorate controls of both sexes were in the middle of the range defined by the NTP control groups. Up to the highest perchlorate dose tested, 10 mg/kg-day, serum T3 and T4 levels were well within the ranges defined by the means of the NTP control groups.

Possible inter-strain differences in T3 levels cannot be excluded, although any such difference are unlikely to be of consequence. With respect to T4 levels, the available data do not support the hypothesis that inter-strain differences are significant. Sprague-Dawley rats were used in the 90-day, drinking-water exposure study of perchlorate, while Fisher-344 (F344) rats were used in all NTP studies reporting T3 levels and in fourteen of the studies reporting T4 levels; Sprague-Dawley rats were used in the other two. The latter two studies reported mean serum T4 levels of 3.7 and 3.9 μg/dl in males and 4.7 and 5.0 μg/dl in females. T4 levels in the NTP Sprague-Dawley males fell at the low end of the NTP range, in between the levels observed for the 1 and 10 mg/kg-day perchlorate groups. By contrast, T4 levels in the NTP Sprague-Dawley females were near the middle of the NTP range, above the level observed in the zero-dose perchlorate group. These observations on the two NTP Sprague-Dawley control groups suggest that this rat strain is likely to demonstrate patterns of T3 and T4 variability similar to those observed for the larger, F344 rat-dominated NTP control data base.

In summary, the historical data base provides evidence *against* the assumption that in rats treated for 90 days with drinking water containing perchlorate at levels as high as 10 mg/kg-day, the resultant depression in T3 and T4 should be considered as physiologically abnormal or adverse.

E. Motor Activity Measurement in the Neurodevelopmental Study

It should be kept in mind that, as indicated on p. 5-37 of the External Review Draft, the non-significant increase in motor activity on PND14 in the pups of dams receiving the lowest-dose, 0.1 mg/kg-day, occurred "in only one gender on only 1 day out of 4 test days" and that "the effect seen

in the males on PND14 may indeed be a Type I error and would not be found again if this experiment was repeated." It is reasonable for EPA/NCEA to request that the trial be performed again, but the lack of dose-response observed among the 0, 0.1 and 1.0 mg/kg-day groups indicates that the most likely outcome is no effect of treatment at doses below 3 mg/kg-day.

F. Recommendations for a Critical NOAEL Based on the Animal Data

1. Increased Size of the Corpus Callosum on PND12

Although the thyroid is certainly the principal target organ for the actions of perchlorate, it is perhaps a mistake to conclude that the critical effect can be found by looking at the thyroid or thyroid hormones directly. The thyroid apparently functions quite well (*i.e.*, in an adaptive fashion) in weanling rats given perchlorate for 90 days at doses up to 10 mg/kg-day, the highest dose tested; this is true also for the PND5 pups of rat dams given perchlorate at 10 mg/kg-day and below. In the latter (neurodevelopmental) study, recovery from increased thyroid follicular-cell hypertrophy in the 10 mg/kg-day group occurred by PND22 when perchlorate treatment was stopped on PND10 (see Table 1, above). From Figs. 5-11 and 5-12 of the External Review Draft (pp. 5-33 and 5-34) one can see that in PND5 rats, T3 and T4 were decreased approximately 60% and 30%, respectively, at a perchlorate dose of 3 mg/kg-day, while TSH was increased approximately 20% at a perchlorate dose of 10 mg/kg-day (Fig. 5-13, p. 5-35). It is conceivable that some developing organs might be affected by thyroid hormone changes of this magnitude during development. For example, it is possible that the 27% increase in the size of the corpus callosum in males and females combined (observed on PND12 in the pups of rats given perchlorate at 10 mg/kg-day) is secondary to altered thyroid hormone levels. Although the significance of this finding is unclear, it seems reasonable that "EPA considers a 27% increase in the size of any brain region to be a potentially adverse effect [ref.]" (p. 5-26). The LOAEL and NOAEL derived by EPA/NCEA from this study, 10 mg/kg-day and 3 mg/kg-day, respectively, constitute an appropriate departure for deriving an RfD.

Note, however, that Argus Laboratories' neurodevelopmental study is a poor model for the effects of chronic perchlorate administration on the developing fetus. Perchlorate exposure began on GD0, thus ensuring that the rat dams (and thus their embryonic fetuses) experienced the shock of shifting serum levels of thyroidal hormones at a very critical time. If the dams had begun perchlorate treatment several weeks prior to mating, it seems likely that the changes in thyroid hormone levels, thyroid histology, and corpus-callosum size observed on PND5 would have been significantly muted, perhaps to the point of insignificance. In this context, it is interesting that one study has found that the fetal rat brain is able to maintain T3 homeostasis to a greater extent than other fetal tissues under the stress of variations in the maternal supply of iodine, T3, or T4 (Morreale de Escobar *et al.*, 1992).

2. Immunotoxicity Results in Mice

The results of the ongoing immunotoxicity study should be evaluated carefully before proceeding with the derivation of a new RfD. The results on macrophage phagocytosis are too inconsistent and too transitory to form the basis of an RfD. According to the External Review Draft, results from a test of humoral immunity (antibody response to SRBC antigen) are anticipated by the date of the peer-review workshop, while repeats of several tests of host resistance (to *L. monocytogenes* and B6F10 tumor cells) are not expected to be complete before June 1999.

III. Utilizing Human Data and Rat/Human Comparisons in the RfD Derivation

A. Ongoing Exposure Studies

One flaw of the External Review Draft is its failure to allow for existing health effects data gathered in occupational and clinical studies of perchlorate exposures. A more important flaw is that the revised RfD was derived during the time that two pertinent exposure studies were in the planning stages (or had been initiated). The Air Force Research Laboratory (AFRL) is currently conducting single-dose and 14-day exposure studies of the kinetics of perchlorate inhibition of thyroidal iodide uptake in rats. Dr. Lewis Braverman of Brigham and Women's Hospital, a well-known expert on the human thyroid, is in the process of conducting an exposure study in human volunteers. The Braverman study is examining thyroidal iodide uptake, serum levels of thyroid hormones, and the elimination kinetics of perchlorate at doses considerably lower than those hitherto tested in humans. Because perchlorate appears to exhibit nonlinear elimination kinetics, it is important to obtain animal and human toxicity health-effects data at doses as close as possible to the environmentally relevant range of exposures (Goodman, 1998). It is also important to be able to compare the inhibition of iodide uptake and the kinetics of perchlorate elimination in rats with those in humans. The data gathered by AFRL and Dr. Braverman should facilitate this comparison. The AFRL studies in rats and the Braverman study in humans are expected to conclude their data-gathering phases in February. The scientific basis of the revised RfD for perchlorate would be greatly strengthened by delaying the risk assessment process until the data from these studies can be taken into consideration.

B. Thyroid Homeostasis

It is well known that humans are able to maintain normal thyroid function over a wide range of iodine intake. This manifests, for example, in a narrower distribution of T4 values in normal humans than in control rats. In a study of 115 nongoitrous, Greek subjects exhibiting a broad spectrum of iodine intakes (*i.e.*, with urinary I/creatinine ratios varying from <50 to >250 μg/g), nearly all serum T4 values were in the 110 to 130 nM range (Moulopoulos *et al.*, 1988). This yields an estimated human T4 variation of approximately 15%, which is to be compared with the two- to three-fold variation in T4 for control rats in 13-week NTP studies (Table 1). Simply put, the rat is expected to be more sensitive than the human to fluctuations in dietary iodine and to agents which affect thyroid hormone levels. EPA/NCEA would do well to explore these established differences before deciding on the appropriate uncertainty factors to use in deriving a revised RfD from a rat LOAEL or NOAEL.

IV. References Not Cited in the External Review Draft

Goodman, G. 1998. "Perchlorate Pharmacokinetics: Critical Review and Evaluation of Relevance to Low-Level Exposures in Humans." September 1, 1998. Submitted to EPA/NCEA.

Morreale de Escobar, G., Calvo, R., Obregon, MJ, Escobar del Rey, F. 1992. Homeostasis of brain T3 in rat fetuses and their mothers: Effects of thyroid status and iodine deficiency. *Acta Med. Austriaca* Suppl. 1: 110-116.

Moulopoulos, D.S., Koutras, D.A., Mantzos, J., *et al.* 1988. *J. Clin. Invest.* 11: 437-439.

Table 2. Paired Comparisons of Thyroid Hormone and Thyroid Histopathology Data as Reported in Figures 6-3 to 6-16 of the External Review Draft

Figure	Comparison	Data Description/Citation (as given in the External Review Draft)	Perchlorate Doses (mg/kg-day)
6-3	T3 *vs.* T4; T4 *vs.* TSH	14-day rat Caldwell *et al.* (1995); Channel (1998a); Crofton (1998a)	0, 0.11, 0.25, 1.1, 2.6, 4.6, 11.5, 22.5
6-4	T4 rank order *vs.* severity rating for follicular hypertrophy; T4 rank order *vs.* severity rating for decreased follicular lumen size	14-day rat study Caldwell *et al.* (1995); Channel (1998a); Crofton (1998a)	0, 0.11, 0.25, 1.1, 2.6, 4.6, 11.5, 22.5
6-5	TSH rank order *vs.* severity rating for follicular hypertrophy; TSH rank order *vs.* severity rating for decreased follicular lumen size	14-day rat Caldwell *et al.* (1995); Channel (1998a); Crofton (1998a)	0, 0.11, 0.25, 1.1, 2.6, 4.6, 11.5, 22.5
6-6	T3 *vs.* T4; T4 *vs.* TSH	14-day and 90-day rat, combined data Springborn Laboratories, Inc. (1998)	0, 0.01, 1.0, 10, 30, 100
6-7	T4, TSH rank order *vs.* severity rating for hypertrophy/ hyperplasia	14-day and 90-day rat, combined data Springborn Laboratories, Inc. (1998)	0, 0.01, 1.0, 10, 30, 100
6-8	T3 *vs.* T4; T4 *vs.* TSH	14-day rat Springborn Laboratories, Inc. (1998)	0, 0.01, 0.05, 0.2, 1.0, 10
6-9	T4, TSH rank order *vs.* severity rating for hypertrophy/ hyperplasia	14-day rat Springborn Laboratories, Inc. (1998)	0, 0.01, 0.05, 0.2, 1.0, 10
6-10	T3 *vs.* T4; T4 *vs.* TSH	90-day rat Springborn Laboratories, Inc. (1998)	0, 0.01, 0.05, 0.2, 1.0, 10
6-11	T4, TSH rank order *vs.* severity rating for hypertrophy/ hyperplasia	90-day rat Springborn Laboratories, Inc. (1998)	0, 0.01, 0.05, 0.2, 1.0, 10
6-12	T3 *vs.* T4; T4 *vs.* TSH	F1 rat pups on PND5 (neurodevelop.) Argus Research Laboratories, Inc. (1998a); York (1998c); Channel (1998b); Crofton (1998f)	0, 0.1, 1.0, 3.0, 10
6-13	T4 rank order *vs.* severity rating for follicular hypertrophy; T4 rank order *vs.* severity rating for decreased follicular lumen size	F1 rat pups on PND5 (neurodevelop.) Argus Research Laboratories, Inc. (1998b); York (1998c); Channel (1998b); Crofton (1998e,f)	0, 0.1, 1.0, 3.0, 10
6-14	TSH rank order *vs.* severity rating for follicular hypertrophy; TSH rank order *vs.* severity rating for decreased follicular lumen size	F1 rat pups on PND5 (neurodevelop.) Argus Research Laboratories, Inc. (1998b); York (1998c); Channel (1998b); Crofton (1998e,f)	0, 0.1, 1.0, 3.0, 10
6-15	T3 *vs.* T4; T4 *vs.* TSH	F0 rabbits on GD29 (developmental)	0, 0.01, 1.0, 10, 30, 100
6-16	T4, TSH rank order *vs.* severity rating for follicular hypertrophy	F0 rabbits on GD29 (developmental)	0, 0.01, 1.0, 10, 30, 100

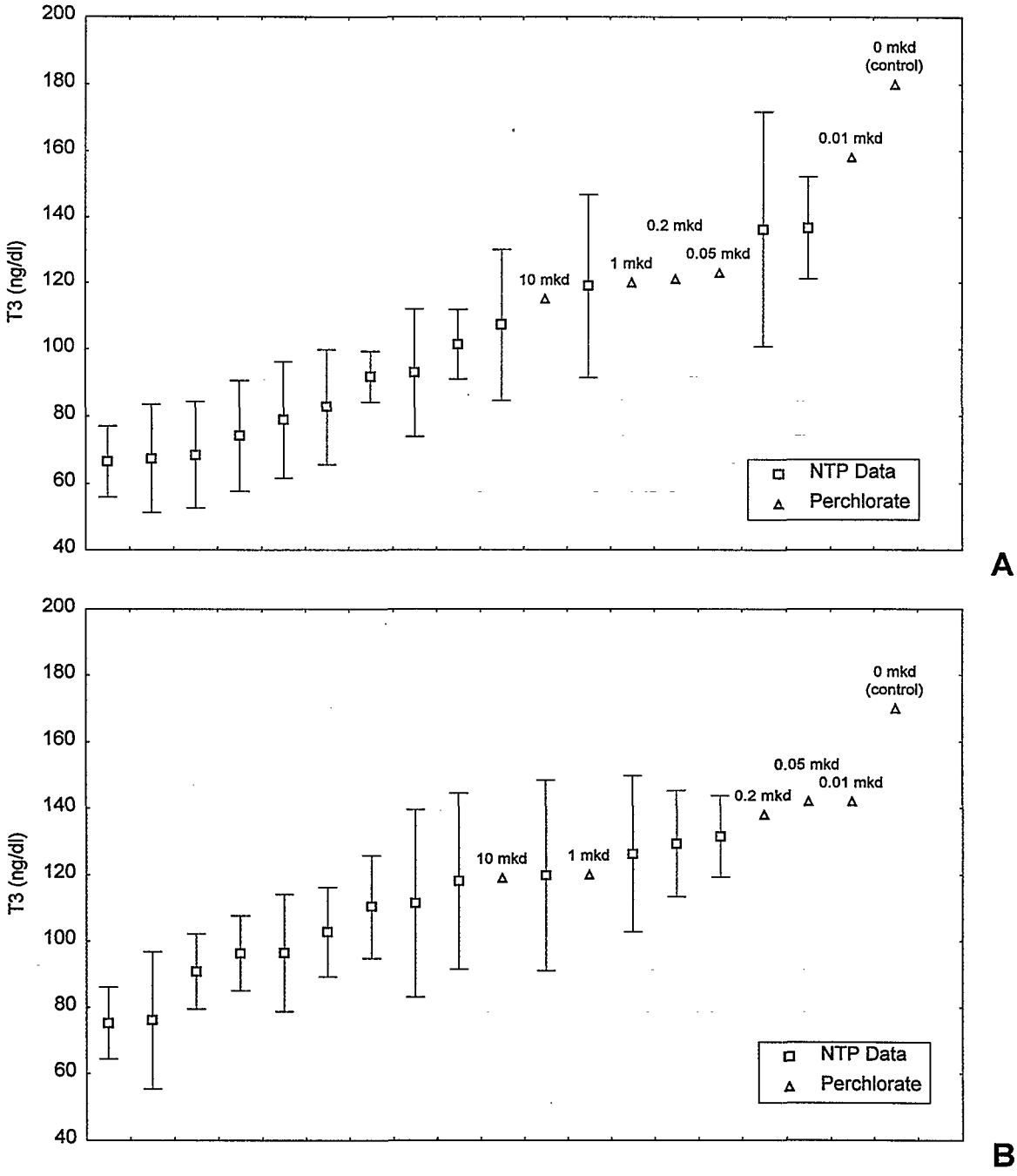

Figure 1. Serum total T3 Levels in male (**A**) and female (**B**) control rats at terminal sacrifice in 13-week NTP bioassays: Rank-order comparison with 90-day data in male (**A**) and female (**B**) rats from the 1998 subchronic exposure study of perchlorate in drinking water. *NTP data:* mean values and standard deviations for the control groups in all thirteen bioassays for which reliable serum T3 measurements are available; courtesy of Dr. Greg Travlos, NIEHS. *Perchlorate data:* mean values; from Springborn Laboratories, Inc., as reported in Fig. 5-6 of the External Review Draft. *Abbreviation:* mkd, mg/kg-day.

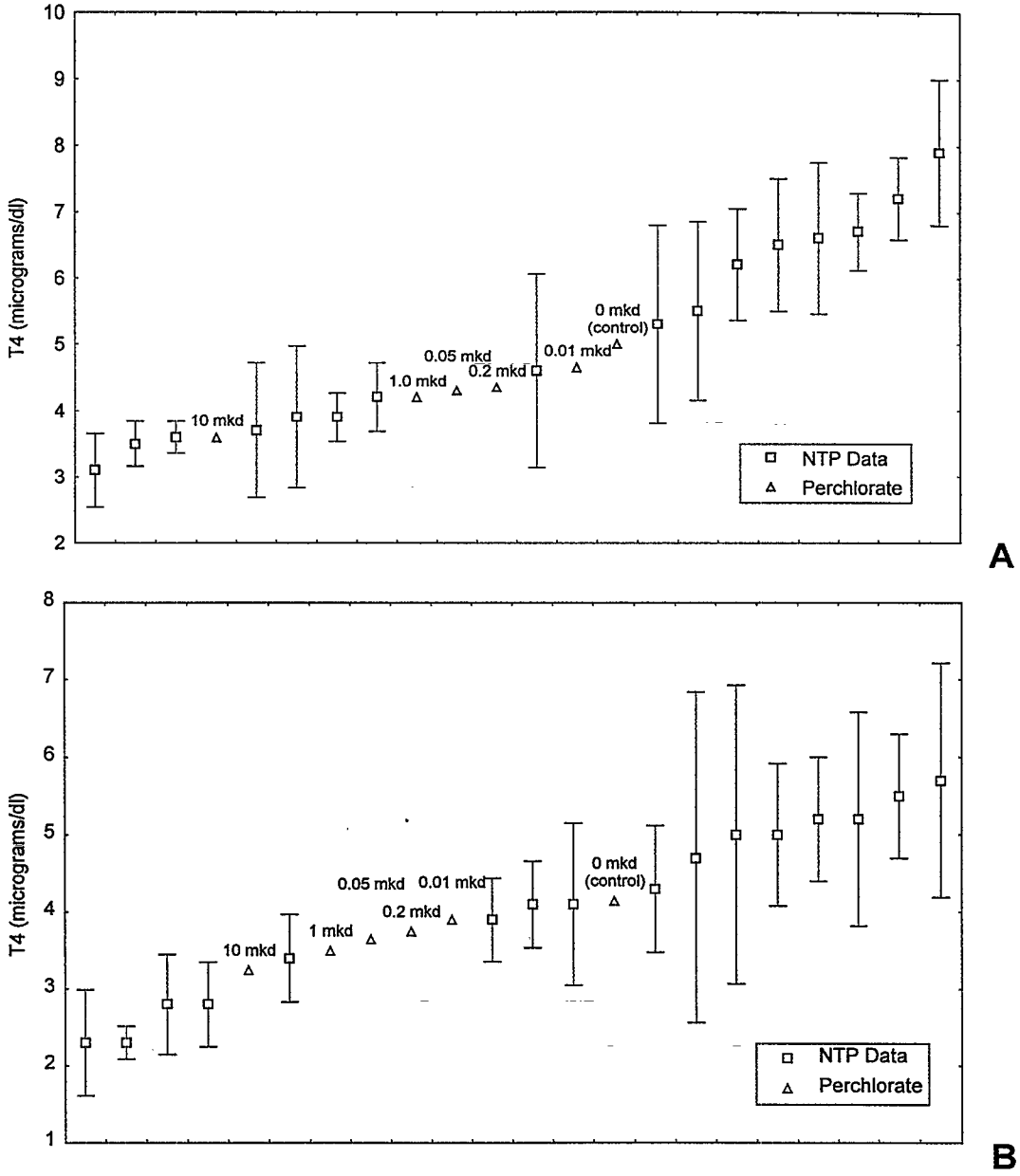

Figure 2. Serum total T4 Levels in male (**A**) and female (**B**) control rats at terminal sacrifice in 13-week NTP bioassays: Rank-order comparison with 90-day data in male (**A**) and female (**B**) rats from the 1998 subchronic exposure study of perchlorate in drinking water. *NTP data:* mean values and standard de*via*tions for the control groups in all sixteen bioassays for which reliable serum T4 measurements are available; courtesy of Dr. Greg Travlos, NIEHS. *Perchlorate data:* mean values; from Springborn Laboratories, Inc., as reported in Fig. 5-8 of the External Review Draft. *Abbreviation:* mkd, mg/kg-day.